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AND LESSER SCAUP DUCKS**

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ACCUMULATION AND EXCRETION OF CI¹⁴ DDT IN MALLARD AND LESSER SCAUP DUCKS¹

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Abstract: The ingestion, metabolism, storage, and excretion of radio-labeled chlorine-36 DDT in wild mallard ducks (*Anas platyrhynchos*) and lesser scaup ducks (*Aythya affinis*) was investigated during 1964 and 1965. A single application of isotope-labeled pesticide was made to a 4-acre marsh at the rate of 0.2 lb/acre in 1964. A total of 112 ducks was subsequently introduced to the treated unit and collected after various exposure periods. Twenty-one tissues and organs from each duck were assayed for DDT residues using liquid scintillation spectrometry, electron capture gas-liquid chromatography, and thin-layer chromatography. A total of 3,760 experimental tissue samples from the 112 ducks and 830 background samples from 100 ducks were processed. Residues of DDT were found at some time in all tissues tested except the testes of lesser scaup. Lesser scaup thyroids, spleen, testes, and ovaries contained no detectable residues during the second year. The compounds DDE, DDD, and *p,p'*-DDT were most common, but DDE was the predominant metabolite found throughout the 2-year period. DDDU was recovered from the liver and brain of both species. Metabolite concentrations are given for tissues sampled. Definite relationships exist between the type of food ingested (plant or animal) and DDT residues. This is reflected in the higher residue levels in scaup tissues. Some of the highest residue concentrations were found in leg fat (mallard 32.8 ppm; scaup 67.2 ppm) and neck fat (mallard 43.8 ppm), and in the uropygial gland (mallard 31.7 ppm; scaup 36.8 ppm), and adrenal glands (mallard 14.8 ppm; scaup 16.3 ppm). Dynamics of equilibrium storage and excretion of DDT residues were also observed.

The purpose of this 2-year field study was to describe details of ingestion, metabolism, storage, and excretion of chlorine-36 radio-labeled DDT in two species of ducks. Residues of DDT were studied in wild-trapped mallard and lesser scaup ducks following a single pesticide application. Pesticide analyses were conducted following different introduction and exposure periods in the treated marsh in order to fulfill the following objectives:

1. To determine the amount of residue present in contents of the gastrointestinal tract.
2. To identify metabolites found within specific tissues.
3. To determine the total amount of labeled DDT and its metabolites present in organ systems.

4. To compare pesticide residue dynamics in mallard and lesser scaup.

My appreciation is extended to T. J. Peterle for the valuable advice and supervision given during the project.

FIELD METHODS

This study was conducted concurrently with another by Meeks (1968). The research site for both projects was a fenced marsh area at Winous Point Shooting Club on Sandusky Bay near Port Clinton, Ohio.

Application of DDT

Labeled DDT was applied to the 4-acre marsh on July 7, 1964, with a helicopter equipped with a rotary hopper applicator. Attapulgite granules (20/35 mesh AA-RVM Attachay) were used as carrier and applied at the rate of 100 lb/acre. A total of 0.2 lb/acre of technical DDT was mixed with 3.9 mc of labeled DDT, dissolved in xylene and mixed with the granules.

During application, the helicopter re-

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leased the formulation at 3 to 6 ft above the water. The downdraft from the rotors forced the granules directly into the water with no lateral outwash onto adjacent areas. Air samples taken on the surrounding dike indicated no drift.

Experimental Birds

Wild ducks used in the study were trapped from sites in Ohio, Michigan, and Canada. Mallards and lesser scaup were selected as test species because of their differing feeding habits. The mallard consumes mainly vegetation (62.4-90.5 percent, Goodman and Fisher 1962:25). Rogers and Korschgen (1966:260-262) reported 82-90 percent of the lesser scaups' diet to be animal material, while Cottam (1939:43-45) stated that adult scaup consume 41 percent and juveniles 90 percent animal matter.

Representative samples of the two species from all sources were analyzed for background radiation and pre-test body burdens of pesticides. Background determinations were also made during various times while wing-clipped ducks were detained in holding pens.

Following the pesticide application, 112 wild-trapped ducks were intermittently released, exposed for various time periods and collected from the treated marsh area. Individual ducks were identified with patagial tags following the procedure of Anderson (1963). Tags of this type provided a satisfactory marking for a maximum of 6 months on mallards, but the plastic-fabric streamers on scaup frayed considerably after 3 months.

After the exposure period was completed, ducks were shot and immediately placed on dry ice. Specimens were transferred to a freezer and remained frozen until dissected.

Predetermined uniform sample sizes were not obtained, and thus, statistical analysis

is incomplete. Adverse weather, dense marsh cover, secretive behavior, and predation predisposed our original plan and unequal samples resulted.

LABORATORY METHODS AND MATERIALS

Chlorine-36 DDT

The labeled DDT was purchased from the Radiochemical Centre, Amersham, Buckinghamshire, England. Our shipment was analyzed as follows: 85.3 percent pp'-isomer; 11.0 percent op'-isomer; and 4 percent unidentified. The location of the chlorine-36 was in the para and para-prime positions on the DDT molecule. These positions have unique analytical significance since the ring structure of the molecule is not broken during metabolism, and therefore, known DDT metabolites remained isotope-labeled. Other valuable characteristics of chlorine-36 are the energy (0.714 mev) and the half-life of 4.4×10^3 years. The relatively high energy of the negative beta particle assures more efficient radioassay, and the long half-life provides a tag which is constant over the term of the project.

Numerous metabolites have been identified as a result of biodegradation of DDT. Abbreviations and chemical nomenclature (after Hayes 1965) for isomers and metabolites found during this project are listed below:

DDT	2, 2-bis (p-chlorophenyl) 1, 1, 1-tri chloroethane
DDD (TDE)	2, 2-bis (p-chlorophenyl) 1, 1-dichloroethane
DDDE	2, 2-bis (p-chlorophenyl) 1, 1-dichloroethylene
DDMU	2, 2-bis (p-chlorophenyl) 1-chloroethylene

In this study, I considered any of these isomers or metabolites that were physically

Table 1. Duck tissues and organs sampled and anatomical site of sample.

TISSUE OR ORGAN	SITE OF SAMPLE	TISSUE OR ORGAN	SITE OF SAMPLE
Breast feathers	All feathers within a 1 square inch from center of breast.	Lung	Any available tissue.
Breast skin	One square inch of plucked skin from which feathers were obtained.	Spleen	Entire organ.
Uropygial gland	Both lobes removed intact complete with plumes.	Kidney	Either kidney homogenized and subsampled.
Breast muscle	One cubic inch of pectoralis major from either side of the sternal keel.	Brain	Cerebral hemispheres, optic lobes, cerebellum and medullary bulb homogenized and subsampled.
Heart muscle	Entire ventricular wall.	Adrenal gland	Entire organ; no tissue available for ECGC.
Gizzard muscle	½ inch lateral cross-section including keratin inner lining.	Thyroid gland	Entire organ; no tissue available for ECGC.
Proventriculus	Entire organ after evacuation and washing.	Testes	Entire organ or subsample depending upon seasonal variation in size.
Small intestine	Duodenal loop after removal of pancreas and evacuation of contents.	Ovaries	Entire organ or subsample depending upon seasonal variation in size.
Large intestine	Intestine between ceca and cloaca after evacuation and washing.	Fat	Collected whenever available depending upon seasonal variation; deposits sampled from neck, subcutaneous of leg and breast, post-ventral body, mesenteric, heart and gizzard. Post-ventral body fat is located in fascia overlying internal and external abdominal oblique muscles.
Pancreas	Entire (dorsal, ventral, and splenic lobes) organ homogenized and subsampled.		
Liver	Lateral cross section through mid-right and left lobes homogenized and subsampled.		
Gall bladder	Entire bladder plus bile contained within.		

accumulated in tissues or formed by biochemical processes as *DDT residues*.

Sample Preparation

Ducks were transferred from the freezer to a refrigerator (3 C) to thaw. When possible, 21 tissues (Table 1) were dissected from each duck and analyzed by liquid scintillation spectrometry and gas-chromatography.

I macerated each sample and used a subsample of less than 700 mg for scintillation counting. These subsamples were then digested in scintillation grade glass vials using Hydroxide of Hyamine 10-X after Herberg (1960) and Rupkin (1961). The scintillation solution was composed of 2,5-diphenyl-oxazole (PPO) as the primary scintillator

and a secondary scintillator, 1,4-bis-(4-methyl-5-phenyl-oxazole) benzene (Di-methyl POPOP), in spectrograde toluene as recommended by Hayes (1963). A transparent solution was formed by emulsification with 1-2 ml of Triton x-100.

Another subsample weighing not more than 7 g was extracted for chromatography following the method reported by de Fauvert Maunder et al. (1961). Thin-layer chromatographic extracts were further cleaned-up by the sulfonation procedure used by Peterson and Robison (1964).

These sample weights represented the maximum limits for satisfactory tissue preparation and optimum machine operation in this study.

Routine Radioassay

All 3,200 tissue samples were analyzed with a Packard Tri-Carb Liquid Scintillation Spectrometer (LSS), Model 3003, equipped with an automatic external standard. The spectrometer freezer temperature was maintained at -5°C. Total DDT residues carrying the isotope tag could be assayed but no distinction could be made between the isomers of DDT and the metabolites. The total radioassay count was converted into ppm, on a wet-weight basis. No untagged pesticide or related compound within the sample could be counted with LSS.

Gas and Thin Layer Chromatography

Electron capture gas chromatograph (ECGC) was used to assay 560 selected samples for quantitative and qualitative purposes. The uropygial gland, breast muscle, liver, and brain were analyzed regularly. These tissues were selected because they were consistently available in ducks, and because they represent, to a certain extent, four tissue types that have been reported most commonly in the literature. In addition, most of the other tissues were analyzed in 37 birds during the first year.

I made all injections into a Barber Colman Gas Chromatograph Series 5,000 equipped with an electron capture tritium detector and a 6-ft glass column. The column packing consisted of a support of Anakrom-ABS (60/80 mesh) with the liquid phase being 1.5 percent (by wt) SE 52. After packing the column, it was conditioned with injections of high concentrations of technical DDT and metabolites (Shuman and Collie 1963) in order to initially fill active sites on packing materials.

A carrier gas of pre-purified nitrogen was used at a flow rate of 100 ml/min.

Operational temperatures were set as follows: flash heater (injector) 230°C, column 21°C, and detector 225°C.

The isomers of DDT and any metabolites that capture electrons could be distinguished. However, no distinction was made between the labeled components of DDT and those not tagged since ECGC registered all DDT-related compounds from any source that was present in the sample.

The distinction between the isotope-tagged pesticide metabolite and those not tagged was made by using autoradiographs of thin-layer chromatography (TLC) plates. Pre-coated Eastman Chromagram® non-fluorescence sheets and thin-layer apparatus was used for all thin layer chromatograms. Reagents used in development of chromatographs are described by Kovacs (1963). R_f and R_{ppm} values were used to identify all compounds. Then autoradiographs of each thin-layer plate using no-screen x-ray film permitted identification of those thin-layer spots containing the isotope. Gas chromatographic findings were confirmed in this way.

RESULTS

Background Tissues

Any duck or duck tissue that was not exposed to the research area comprised background samples.

Ducks used in the study were collected from five different geographical areas, and a wide variation in pesticide background values was apparent between birds of different collection sites. In this report, the background values which were subtracted from experimental values were always from birds of the same geographical source and history.

I evaluated a total of 710 non-exposed tissues sampled from 14 ducks to determine the background for radioassay (LSS). Since

Table 2. Mean DDT residues (ppm) found in alimentary tracts of wild ducks exposed for various periods on a 4 acre marsh treated once with 0.2 lbs DDT per acre 1964-1965.

Gizzard or tissue	TOTAL Residues (LSS)	Metabolites (ECCG)*		
		n	DDE	DDD p,p'-DDT
Mallard Gizzard	n = 53 2.5	7	T ^b	ND ^c
Small Intestine	1.1	7	0.3	0.3
Large Intestine	1.1	3	0.3	ND
Lesser Scaup Gizzard	n = 47 0.8	4	1.8	ND
Small Intestine	0.0	13	1.8	0.0
Large Intestine	0.2	10	1.2	ND

LSS = Liquid scintillation spectrometry—all metabolite residues.

ECCG = Electron capture gas chromatography—separate metabolites.

* DDDMU not detected.

^a 0.01-0.09 ppm.

^b Not detected.

only the maximum level of radioactivity; in each tissue was of interest, the one-tailed *t* distribution was used to calculate the confidence intervals ($P < 0.01$) for background values. Coefficients of variation for LSS background samples ranged from 3.29 to 38.05 percent for various tissues with no significant differences ($P \geq 0.05$) between species.

Concentrations of background pesticide residues were also determined by ECCG. Samples of 1-10 tissues from 15 ducks were prepared. In nearly all cases where residues were found, DDE was the predominant metabolite and was therefore used for evaluation of ECCG residue variability within tissues. The coefficient of variation of DDE residues obtained from ECCG background tissues ranged from 42.39 to 95.62 percent in mallards and from 30.25 to 103.74 percent in scaup.

The different geographical sources of ducks account for some of this variation. For example, the DDE values for the metapygial gland of scaup from British Columbia ranged from 0.56 to 20.70 ppm, while residue in scaup trapped at Pt. Mouillee, Michigan, ranged from 4.18 to 10.62 ppm. The DDE concentrations in the metapygial gland of background mallards trapped at Port Clinton, Delaware, and Clyde, Ohio, were 0.41 to 2.97 ppm, 0.39 to 7.07 ppm, and 0.33 to 0.73 ppm, respectively.

Considering all background tissues examined per bird, no mallard or scaup was found to be free of DDT residues.

Ingestion and Excretion

Details of ingestion and excretion of Cl³⁶ DDT residues were studied by assay of contents of the gizzard and small and large intestine. No food materials were in the proventriculus. Trends in mean residues found in the gastrointestinal contents over the 2-year period were different between species (Table 2).

Expected differences in food choice between the two species are apparent from the gizzard content analysis of 76 birds (Table 3). Feeding differences are also reflected in different residue trends in Table 2. Whenever the occurrence of snails (*Urticaria* sp.) in the gizzard exceeded 23 percent, DDT residues in the gizzard were less than those found in the contents of the small intestine. Gizzard content residues were greater when plant materials formed a high percentage of the diet. Only one exception to this trend appears and that is in mallards during the first year. In mid-July, soon after treatment, mallards exposed for 6 hours contained 80 percent snails in their gizzard and had residues 1.4 times higher than small intestine contents (0.2 ppm). Snails still present in the gizzard were completely intact, and therefore,

Table 3. Gizzard contents (percent occurrence) of ducks exposed to a 4 acre marsh treated with 0.2 lbs DDT per acre.

Food Intake	Mallard n = 30			Lesser Scaup n = 10		
	1964	1965	Both years	1964	1965	Both years
Snails						
<i>Helixoma</i> sp.	18.7			7.0	20.0	11.1
Beetle				3.7	2.3	3.7
<i>Coleoptera</i>					3.7	3.3
Seed					3.7	3.3
<i>Gramineae</i> sp.					2.7	3.3
Total animal	18.7	3.7	9.3	23.4	16.5	21.0
Coontail ^a						
<i>Ceratophyllum</i> sp.					7.4	5.7
Duckweed ^b						
<i>Lemna</i> sp.		22.2	14.1	18.5	2.7	9.7
Pondweed ^b						
<i>Potamogeton</i> sp.	25.0	22.2	23.2	7.4	17.1	12.3
Water Lily ^b						
<i>Nymphaea</i> sp.	6.3	7.4	7.0	3.7		1.6
Bulrush ^b						
<i>Scleria</i> sp.	12.5	7.4	9.3	3.7	11.1	9.7
Smartweed ^b						
<i>Polygonum lapathifolium</i>	25.0	22.2	23.2	22.2	20.6	21.1
<i>Polygonum</i>						
<i>pennsylvanicum</i>		3.7	2.3		2.7	1.6
Rose Mallow ^b						
<i>Hibiscus palustris</i>					3.7	8.6
Fabaceae ^b						
<i>Amnicariae</i>	12.5	11.2	11.0		5.7	3.2
Total plant	81.3	96.3	90.7	80.0	83.2	75.4

^a Vegetation.^b Seeds.

no small tissue had advanced into the intestine. The higher trend of residues in the small intestine would have possibly held true had the ducks been allowed to live longer.

During the study, DDE was the only metabolite found in gizzard contents, while the contents of the small intestine contained DDE, DDD, and *p,p'*-DDT. Contents of the large intestine were DDE and *p,p'*-DDT. The presence of DDD in the small intestine and absence in large intestine indicates absorption. Perhaps all metabolites are absorbed in the duodenum. The changes in the mean DDT residue in the alimentary tracts (Table 2) of both species also suggest absorption by this organ.

Metabolism and Storage of Residues

Radioassay data from tissues of both species were combined and analyzed regardless of exposure periods (Tables 4 and 5). Correlation coefficients in Table 5 show relationships between total DDT residue concentrations of 11 tissues. Significant correlations exist between all tissue residues except those associated with breast skin, breast feathers, and thyroids. Similar correlations between body and wing tissues were reported by Dindal and Peterle (1968).

Species variation in total DDT residues (LSS), DDT metabolites (ECCG), and trends related to exposure periods are given for all tissues (Tables 6-11). Total metabolite values from ECCG analysis do not al-

Table 4. Total DDT residues (ppm) in tissues of mallard and lesser scaup ducks: combined data, 1964-1965. (Radioassay analysis, LSS). Exposure periods 0 hr-130 days.

Tissue	MEAN \pm STANDARD ERROR
Uropygial glands	4.12 \pm 0.91
Adrenal glands	2.60 \pm 0.02
Breast feathers	1.55 \pm 0.32
Liver	1.35 \pm 0.22
Breast skin	1.23 \pm 0.31
Pancreas	0.5 \pm 0.11
Gonads	0 \pm 0.10
Kidney	0.45 \pm 0.07
Thyroid gland	0.44 \pm 0.23
Brain	0.41 \pm 0.08
Breast muscle	0.28 \pm 0.03
Wings (muscle, bone, skin)	0.51 \pm 0.09

Ducks n = 101. Those n = 1248.

ways correspond exactly with total DDT residues obtained with LSS. This difference is due to the greater variability of ECCC background versus a relatively low LSS background variability as was indicated by coefficient of variation.

Only one experimental tissue, the testis of lesser scaup, was free of detectable DDT residue during all exposure periods. Residues were found in all other tissues at some time during the study.

Generally, the lowest residues (LSS) in all samples were found the second year

(1965) in ducks introduced 413 days after application and exposed for 30 days. During this period the highest concentration in mallards was 0.61 ppm in the liver and the lowest, none detectable, in breast feathers, breast skin, gall bladder, lung and ovary. In lesser scaup introduced 413 days after application and exposed for 7 days, only two organs contained any detectable residues, the breast muscle 0.43 ppm, and the brain 0.19 ppm.

Some of the highest residue totals appeared in the first 30-day exposure period in 1964, the year of treatment. The highest tissue concentration in mallards was 43.83 ppm in neck fat, the second highest during the first exposure period was 16.31 ppm in uropygial glands, and the lowest in breast muscle ranging from 0.09 to 0.58 ppm. In lesser scaups, the highest residue concentration was 67.23 ppm in subcutaneous leg fat, the second highest concentration was 30.77 ppm in uropygial gland, and the lowest, none detectable, in the testes. With the exception of the scaup testes, none of the samples analyzed from mallards and scaup were totally free of DDT residues (LSS) during the first 30 days of exposure.

Table 5. Correlation (r)^a of total DDT residues in body tissues of wild ducks: Combined data (Radioassay analysis, LSS). n = 104 Ducks, 59 Mallards, 45 Lesser Scaup.

	BREAST FEATHERS	THYROID	BREAST SKIN	LIVER	BREAST MUSCLE	PANCREAS	GONADS	BRAIN	ADRENAL	KIDNEY	UROPYGIAL GLAND
Breast feathers	1.00										
Thyroid	.06	1.00									
Breast skin	.01	.07	1.00								
Liver	.10	.43	.23	1.00							
Breast muscle	-.03	.37	.54	.50	1.00						
Pancreas	.23	.21	.41	.40	.00	1.00					
Gonads	.15	.53	.31	.47	.03	.09	1.00				
Brain	.19	.28	.47	.45	.07	.75	.80	1.00			
Adrenal	.23	.31	.52	.42	.09	.80	.69	.77	1.00		
Kidney	.17	.32	.52	.41	.81	.78	.69	.70	.71	1.00	
Uropygial gland	.15	.53	.49	.52	.80	.80	.84	.82	.82	.82	1.00

^a Significance level ($P < 0.01$) of $r = 0.20$.

Significance level ($P < 0.05$) of $r = 0.20$.

Table 6. DDT residues (means, ppm) in the tissues of mallard ducks exposed to a 4-oz./ac. field treated with 0.2 lb of DDT per acre, 1964. (Analysis: total residues, TSS; Metabolites, ECCC, HCC)

Introduced Days After Application	0	0	0	0	15	15	30	30	68	78	109	109
Days Exposed	9	15	30	63	1/4	45	30	130	92	82	15	51
n (Ducks)	1	4	2	1	4	1	1	1	2	1	2	5
Breast feathers												
Total residues	8.1	0.0	5.0	15.5	2.8	ND*	4.9	2.1	7.0	7.1	2.2	2.1
DDE	ND	ND	-	0.3	-	-	-	-	-	-	-	-
DDD	ND	ND	-	ND	-	-	-	-	-	-	-	-
p,p'-DDT	ND	ND	-	ND	-	-	-	-	-	-	-	-
Breast skin												
Total residues	ND	4.0	13.4	0.5	T	2.0	0.4	ND	0.5	ND	1.0	ND
DDE	LS	2.9	0.0	0.3	-	-	-	ND	0.5	2.7	-	ND
DDD	ND	ND	1.5	NP	-	-	-	ND	ND	0.1	-	ND
p,p'-DDT	ND	4.7	2.7	ND	-	-	-	ND	3.7	ND	-	ND
Uropygial gland												
Total residues	19.3	7.0	12.8	0.0	T	2.1	1.1	-	34.7	14.3	0.7	0.9
DDE	15.5	2.0	3.2	4.7	-	0.1	3.0	-	12.4	7.9	0.4	0.3
DDD	ND	0.4	0.4	ND	-	ND	0.2	-	0.0	ND	0.1	0.1
p,p'-DDT	5.0	4.3	1.3	ND	-	ND	1.8	-	17.0	ND	2.7	0.4
Breast muscle												
Total residues	0.1	0.0	0.2	ND	T	ND	T	ND	0.7	0.1	ND	T
DDE	-	0.4	0.1	T	-	T	0.2	T	0.4	-	0.4	0.3
DDD	-	0.1	0.1	ND	-	ND	ND	ND	0.1	-	0.1	0.1
p,p'-DDT	-	ND	T	ND	-	ND	ND	ND	0.2	-	2.7	0.4
Heart muscle												
Total residues	1.0	0.8	1.5	ND	0.2	0.0	ND	T	1.1	0.6	ND	0.1
DDE	0.9	0.2	0.4	0.5	-	-	0.2	0.2	0.6	0.3	0.4	0.1
DDD	ND	0.1	0.4	ND	-	-	ND	0.1	0.3	0.1	0.3	ND
p,p'-DDT	0.2	ND	0.2	ND	-	-	ND	ND	ND	0.2	ND	ND
Gizzard muscle												
Total residues	1.5	0.5	1.5	ND	0.2	ND	0.2	0.7	0.5	3.0	ND	0.3
DDE	0.2	0.1	0.1	0.2	-	-	0.1	0.1	T	0.1	T	1.0
DDD	ND	T	ND	ND	-	-	ND	0.1	T	ND	ND	ND
p,p'-DDT	0.1	ND	T	ND	-	-	ND	ND	T	ND	ND	T
Proventriculus												
Total residues	1.2	1.0	0.9	ND	0.2	ND	ND	-	-	ND	0.2	T
DDMU	ND	0.5	ND	ND	-	-	ND	-	-	ND	ND	-
DDE	0.8	0.2	0.2	0.3	-	-	0.2	-	-	0.4	0.2	-
DDD	ND	ND	0.5	T	-	-	ND	-	-	0.1	ND	-
p,p'-DDT	ND	ND	0.2	ND	-	-	ND	-	-	ND	ND	-
Small intestine												
Total residues	2.9	0.0	0.5	0.7	0.0	ND	0.3	1.1	-	-	0.7	0.3
DDE	1.0	0.1	0.1	0.5	-	-	0.1	-	0.1	-	0.1	T
DDD	ND	T	0.1	ND	-	-	ND	-	T	-	0.1	ND
p,p'-DDT	ND	ND	ND	0.1	-	-	ND	-	ND	-	0.4	ND

* DDMU not detected unless indicated.

† Not detected.

‡ Trace 0.01-0.09 ppm.

Table 6. (Continued).

ACCUMULATION AND EXCRETION OF DDT IN MALLARD AND SCAUP • Bindal 83

Table 7. DDT residues (means, ppm) in the tissues of mallard and scaup exposed to DDT per acre, 1964. (Analysis: total residues, TSS per group ducks exposed to a 4-acre marsh treated with 0.2 lb/acre, * EGGC, TLC)

Introduced Days After Application	0	8	15	30	50	50	50	78
Days Exposed	15	30	15	15	30	46	46	17
n (Ducks)	4	4	1	5	6	2	2	5
Breast feathers								
Total residues	1.2	0.0	0.2	1.3	0.5	1.0	3.6	
Three EGGC samples; introduced 0, collected 15; DDE 1.0, DDD ND, <i>p,p'</i> -DDT 0.8								
Breast skin								
Total residues	10.6	8.1	0.8	1.3	1.2	0.3	0.4	
DDE	11.3	4.9	—	—	—	—	—	
DDD	ND	3.0	—	—	—	—	—	
<i>p,p'</i> -DDT	5.4	ND	—	—	—	—	—	
Uropygial gland								
Total residues	30.8	11.4	0.5	3.9	2.3	2.5	2.0	
DDE	20.7	10.1	—	0.7	0.8	0.8	16.0	
DDD	1.1	1.3	—	0.1	ND	ND	0.4	
<i>p,p'</i> -DDT	ND	2.3	—	0.3	ND	ND	4.8	
Breast muscle								
Total residues	2.1	1.1	ND	0.8	0.4	0.5	0.3	
DDE	1.5	0.2	0.1	0.7	0.2	—	1.0	
DDD	T	0.1	T	0.1	ND	—	T	
<i>p,p'</i> -DDT	ND	0.1	ND	0.3	ND	—	1.0	
Heart muscle								
Total residues	3.0	2.1	0.1	1.0	0.3	1.3	0.4	
DDE	—	0.4	—	1.0	—	—	1.9	
DDD	—	0.1	—	ND	—	—	T	
<i>p,p'</i> -DDT	—	0.1	—	1.1	—	—	1.7	
Gizzard muscle								
Total residues	1.0	0.0	0.4	1.3	0.2	0.8	0.2	
DDE	0.1	—	—	0.3	ND	—	0.3	
DDD	ND	—	—	T	ND	—	T	
<i>p,p'</i> -DDT	ND	—	—	0.3	ND	—	0.1	
Proventriculus								
Total residues	2.0	0.9	2.5	0.3	0.5	0.4	0.8	
DDE	—	0.7	—	0.8	0.2	—	0.7	
DDD	—	0.1	—	T	ND	—	T	
<i>p,p'</i> -DDT	—	0.4	—	0.3	ND	—	0.7	
Small intestine								
Total residues	0.8	0.6	1.1	0.2	0.2	0.2	0.1	
DDE	—	0.1	—	0.4	0.1	—	0.3	
DDD	—	T	—	0.2	ND	—	T	
<i>p,p'</i> -DDT	—	T	—	0.3	ND	—	0.1	

* DDMU not detected unless indicated.

† Not detected.

‡ Trace 0.01–0.09 ppm.

Table 7. (Continued).

Table 8. DDT residues (means, ppm) in the tissues of mallard ducks exposed to a 4-acre marsh treated with 0.2 lb of DDT per acre, 1963. (Analyses: total residues, TSS; Metabolites,^a tCCG, TC)

Introduced Days After Application	30	300	300	300	300	300	413	413
Days Exposed	15	30	45	114	15	63	12	30
n (Ducks)	7	4	6	1	0	1	3	7
Breast feathers								
Total residues	0.6	1.8	3.1	ND*	ND	ND	ND	ND
Breast skin								
Total residues	ND	ND	0.2	ND	ND	ND	ND	ND
Uropygial gland								
Total residues	0.2	0.9	0.8	1.2	0.2	0.4	ND	0.5
DDT	1.7	3.4	0.3	-	0.1	0.1	0.1	0.2
DDD	ND	ND	ND	-	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	-	ND	ND	ND	ND
Breast muscle								
Total residues	0.1	0.1	0.1	0.1	T	0.1	0.1	T
DDE	-	-	T	-	T	ND	T	T
DDD	-	-	ND	-	ND	ND	T	ND
p,p'-DDT	-	-	ND	-	ND	ND	ND	ND
Heart muscle								
Total residues	ND	ND	0.2	ND	0.1	0.1	ND	0.1
Gizzard muscle								
Total residues	T	ND	ND	ND	ND	ND	ND	0.1
Proventriculus								
Total residues	0.2	ND	T	ND	0.3	0.5	ND	0.1
Small intestine								
Total residues	ND	ND	0.3	ND	0.0	ND	0.0	0.2
Large intestine								
Total residues	0.4	ND	T	ND	0.0	0.3	ND	0.5
Pancreas								
Total residues	ND	ND	0.2	ND	0.1	ND	ND	T
Liver								
Total residues	ND	0.2	0.2	ND	T	0.0	0.4	0.0
DDMU	ND	ND	ND	-	ND	0.7	ND	ND
DDE	7.6	0.3	0.3	-	ND	0.8	T	ND
DDD	T	ND	ND	-	ND	0.1	T	ND
p,p'-DDT	ND	ND	0.9	-	ND	ND	ND	ND
Gall bladder								
Total residues	ND	T	0.3	ND	ND	ND	ND	ND
Lung								
Total residues	0.1	ND	0.1	ND	0.1	ND	ND	ND
Spleen								
Total residues	ND	ND	ND	ND	0.5	0.5	ND	T
Kidney								
Total residues	0.1	0.1	0.2	ND	0.1	ND	0.1	0.1
Brain								
Total residues	0.1	0.1	0.1	ND	0.1	0.2	0.0	0.1
DDE	4.0	0.21	ND	-	ND	ND	ND	0.1
DDD	ND	ND	ND	-	ND	ND	ND	ND
p,p'-DDT	0.2	ND	ND	-	ND	ND	0.1	ND
Adrenal gland								
Total residues	0.5	0.7	0.3	ND	1.1	ND	0.2	0.4
Thyroid gland								
Total residues	ND	ND	T	ND	0.2	ND	T	0.2

* DDT/DDE not detectable unless indicated.

† Not detected.

‡ Trace 0.01-0.00 ppm.

Table 9. DDT residues (means, ppm) in the tissues of lesser scaup ducks exposed to a 4-acre marsh treated with 0.2 lb of DDT per acre, 1965. (Analyses: total residues, TSS; Metabolites,* ECGC, TLC)

Introduced Days After Application	360	300	300	300	360	360	413
Days Exposed	15	18	30	114	33	97	7
n (Ducks)	0	3	3	1	4	2	1
Breast feathers							
Total residues	0.2	0.8	ND*	ND	ND	2.8	ND
Breast skin							
Total residues	ND	ND	T*	ND	0.1	0.1	ND
Uropygial gland							
Total residues	3.6	1.9	3.9	0.7	0.7	0.7	ND
DDMU	ND	ND	7.9	-	ND	ND	ND
DDE	15.0	11.9	14.4	-	0.9	0.2	0.6
DDD	ND	ND	ND	-	ND	ND	ND
p,p'-DDT	ND	ND	7.9	-	ND	ND	ND
Breast muscle							
Total residues	0.3	0.1	0.1	0.1	0.1	ND	0.4
DDF	0.3	0.3	T	-	T	T	7.2
DDD	ND	ND	ND	-	ND	ND	ND
p,p'-DDT	ND	ND	ND	-	ND	ND	ND
Heart muscle							
Total residues	0.2	ND	0.2	ND	0.2	T	ND
Gizzard muscle							
Total residues	0.1	ND	0.3	ND	ND	T	ND
Proventriculus							
Total residues	ND	ND	ND	ND	ND	0.1	ND
Small intestine							
Total residues	T	ND	ND	ND	ND	ND	ND
Large intestine							
Total residues	0.3	ND	T	ND	0.1	ND	ND
Pancreas							
Total residues	0.1	ND	ND	ND	ND	ND	ND
Liver							
Total residues	0.0	1.4	ND	ND	0.1	ND	ND
DDMU	ND	ND	ND	-	0.5	0.2	-
DDE	1.0	0.9	0.9	-	T	T	-
DDD	ND	ND	ND	-	ND	ND	-
p,p'-DDT	ND	ND	ND	-	ND	ND	-
Gall bladder							
Total residues	ND	ND	0.3	ND	ND	ND	ND
Lung							
Total residues	ND	ND	ND	ND	0.2	0.1	ND
Spleen							
Total residues	ND	ND	ND	ND	ND	ND	ND
Kidney							
Total residues	0.1	0.1	0.1	ND	ND	0.1	ND
Brain							
Total residues	0.1	0.2	0.2	0.2	0.1	0.2	0.2
DDE	1.0	0.6	0.0	-	T	T	0.6
DDD	ND	ND	ND	-	ND	ND	ND
p,p'-DDT	ND	ND	0.0	-	ND	ND	ND
Thyroid gland							
Total residues	ND	ND	ND	ND	ND	ND	ND
Adrenal gland							
Total residues	0.8	0.5	0.0	ND	0.2	ND	ND

* DDMU not detected unless indicated.

† Not detected.

* Trace 0.01–0.00 ppm.

The same metabolites and isomers found in background ducks were found in experimental animals, namely DDDMU, DDE, DDD, and *p,p'*-DDT. No *o,p'*-DDT was found in tissues. The metabolite DDE was generally predominant whenever DDT residues were found. Also, DDDMU when present, was found most often in liver and brain, but occurred once in the tissues of mallard proventriculus and once in lesser scaup uropygial glands.

DISCUSSION

Ingestion

Rate of absorption of residues and residue maxima in tissues appears related to the type (plant or animal) and quantity of food ingested. In both duck species, plant materials are involved in a longer storage and grinding process in the gizzard, while animal matter is moved rapidly from the gizzard into the small intestine. When there is a high proportion of plant materials in the duck's diet, a sporadic pattern of maximum accumulation and excretion of DDT residues is observed. Since plant parts are detained for relatively longer periods in the gizzard, DDT residues associated with plant materials are released into the cavity of the duodenum irregularly and thus, absorption is more sporadic. This pattern was common in mallards. During 1965, when the dominant part of the seaup's diet was pondweed (*Potamogeton* spp.) and smartweed (*Polygonum* spp.) seeds, residue accumulations in body tissues were sporadic. In 1965, when snails made up the greatest part of the seaup's diet, DDT residues accumulated in tissues in a uniform pattern. It appears that the site of absorption is reached more rapidly by animal matter and, within a given duck, the DDT residues accumulate to their maximum levels in all tissues uniformly.

Table 10. DDT residues (mean of wild ducks exposed to a marsh treated with 0.2 lb of DDT per acre, 1964-1965)

Tissue	INTRODUCED DATE AFTER APPLICATION	DAYS EXPOSED	(DUCKS) n	TOTAL RESIDUES (PPM)
<i>Mallard</i>				
Testes	0	0	1	4.9
	0	15	1	2.9
	15	45	1	ND*
	30	130	1	1.8
	109	15	1	ND
	109	51	4	ND
	300	15	7	0.1
	300	30	1	ND
	300	45	2	0.4
	360	15	3	0.2
	413	30	3	0.4
Ovary	0	15	3	1.3
	0	30	2	ND
	15	1/4	4	0.2
	68	92	2	2.7
	78	82	1	ND
	109	25	1	ND
	109	51	1	4.4
	300	30	3	ND
	300	45	4	0.2
	300	114	1	ND
	360	15	3	0.5
	360	65	1	ND
	413	12	3	ND
	413	30	4	ND
<i>Lesser Scaup</i>				
Testes	Exposed 0-35 days Both years n = 31			ND
Ovary	Exposed 15 days 1961 n = 3 Exposed 7-114 days Both years n = 10			ND

* Not detected.

Excretion

The mammalian gall bladder has an important excretory function in returning metabolites to the intestinal tract (Hayes 1965). My data suggest such a process in wild ducks. The dominant metabolites of the mallard gall bladder and contents of the large intestine are DDE, DDD, and *p,p'*-DDT, while only DDE is present in the same organs in lesser scaup. Metabolites of the gall bladder empty into the intestine via the right and left bile ducts

Table 11. DDT residues (ppm) in adipose tissues of wild ducks exposed to a 1-acre marsh treated with 0.2 lb of DDT per acre.

FAT TISSUE	INTRODUCED DAYS AFTER APPLICATION	DAYS EXPOSED	(DUCKS) n	RESIDUES (MEANS)*			
				(LSS) Total	DDE	DDD	p,p'-DDT
<i>Mallard</i>							
Subcutaneous (Leg)	0	15	4	8.9			
	0	30	2	32.8	12.1	1.7	4.6
	300	15	1	0.7			
	360	15	2	ND ^b	3.8	1.1	ND
Subcutaneous (Breast)	0	15	4	2.7			
Heart	0	15	4	8.1			
Mesenteric	0	15	4	4.8			
	0	30	4	5.0	2.4	1.5	0.0
	15	1/1	4	1.1			
	300	45	1	ND			
	360	15	2	ND			
Post-ventral body	0	30	2	12.8	8.2	1.1	2.5
	15	1/4	2	ND			
Neck	0	30	2	43.8			
Gizzard	0	15	2	0.5			
	360	15	2	ND			
<i>Cinnamon Teal</i>							
Subcutaneous (Leg)	0	15	4	67.2			
	0	30	4	33.1	13.3	1.0	0.3
Heart	0	15	4	2.3			
Mesenteric	0	30	4	7.8			
Post-ventral body	0	30	4	11.1			

^a DDDMU not detected.^b Not detected.

at the distal end of the duodenal loop. Assuming that the maximum absorption of metabolites occurs (along with the lipids) at the proximal end of the duodenum, this discharge of metabolites from the gall bladder accounts for the residue present in the contents of the large intestine. The isomer *p,p'*-DDT was not recovered in contents of the large intestine of scaup nor was it found in the gall bladder, which substantiates this idea.

The mean concentrations of residues in alimentary contents were given as 1.1 ppm for both contents of small and large intestine of mallards (Table 2). Similar residue concentrations in small and large intestine contents may be the result of DDT residues

adsorbed on non-digestible cellulose fibers or wax molecules. This suggests that some residues may pass through the gastrointestinal tract without being absorbed by the intestine. Cellulose molecules may provide sites of adhesion of pesticide residues recycled into the intestine through the bile. Also, cholesterol absorption may be inhibited as it was in the chicken (Peterson 1951) by the presence of phytosterol present only in vegetative matter. If DDT residues have any specific affinity for cholesterol, the effect is obvious. Delayed absorption or non-absorption (because of cellulose absorption or phytosterol inhibition) may help account for irregular concentration trends between tissues and lower residue

accumulations in tissues of mallards compared to scaup.

Metabolites Identified

DDT residues, predominantly DDE, were found at some time during the project in each exposed tissue, except the testes of lesser scaup.

Peterson and Robison (1961) reported the intermediate metabolite, DDDMU, from liver of rats. Lesser scaup and mallard livers also contained this compound in 10 different samples during the 2 years. In 1961, from 0.08 to 0.83 ppm were recovered from scaup livers, while 0.17 to 0.20 ppm were present in liver tissue of mallards. The following year, the range in scaup liver was 0.15 to 0.51 ppm and in mallards, 0.73 to 0.88 ppm. The DDDMU range in lesser scaup varied more compared to the range in mallards. Brain tissue samples contained DDDMU only twice during 1961. Mallard brains had 1.38 ppm and scaups, 0.40 ppm. One sample of scaup utropygial glands in 1965 registered 7.90 ppm and a mallard proventriculus once had 0.05 ppm DDDMU in early 1961.

Sites of Metabolism

Perhaps, much of the DDD found associated with marsh plants in the gastrointestinal tracts of the living ducks had been produced from DDT by a great variety of aquatic and intestinal microorganisms. Data gathered by Barker et al. (1965), Kallman and Andrews (1963), Miskus et al. (1965), and Mendel and Walton (1966), substantiate this statement.

The metabolite DDE in gizzard contents probably originated from previous metabolic processes of the animals ingested, from plant cells, or from microorganisms within the gizzard cavity.

It is difficult to specify definite sites of metabolism within duck tissues. Metabo-

lites may be formed within a given tissue and transferred to another, or they may be products of external metabolism (including gastrointestinal) by microorganisms.

Storage

In observing any tissue residues for a given exposure period, maximum peaks were reached and followed by a decrease in concentration. This same pattern occurred even during overlapping exposure periods whether concentrations were higher or lower than readings from ducks that were still present in the marsh from a previous introduction. The maximum pesticide residues recorded during the study for mallard muscle tissues were: heart 1.57 ppm, breast 1.57 ppm, and gizzard 1.50 ppm (Table 7). Similar maxima give an indication of storage equilibrium of muscle tissue for this particular DDT exposure. Hayes (1965) presents experimental results from mammal studies which show that such a storage equilibrium is reached followed by excretion of the pesticide.

Lesser scaup tissues accumulate larger DDT residue concentrations than do mallard tissues during the same length exposure periods independent of the year (Fig. 1). However, this accumulation is short-lived since a more rapid turnover of residues occurs in scaup than in mallards.

Breast Feathers.—The metabolites DDE, DDD, and *p,p'*-DDT found in or on breast feathers of mallards can be explained in two possible ways. First, the DDT residues may be the metabolic products of some aquatic microorganism present in the marsh. Since DDE in mallard feathers was high during periods when duckweed (*Lemna* sp.) was abundant, I assume DDE was associated by absorption to duckweed and was transferred physically as ducks fed among this floating plant. The scattered location of duckweed and the means of

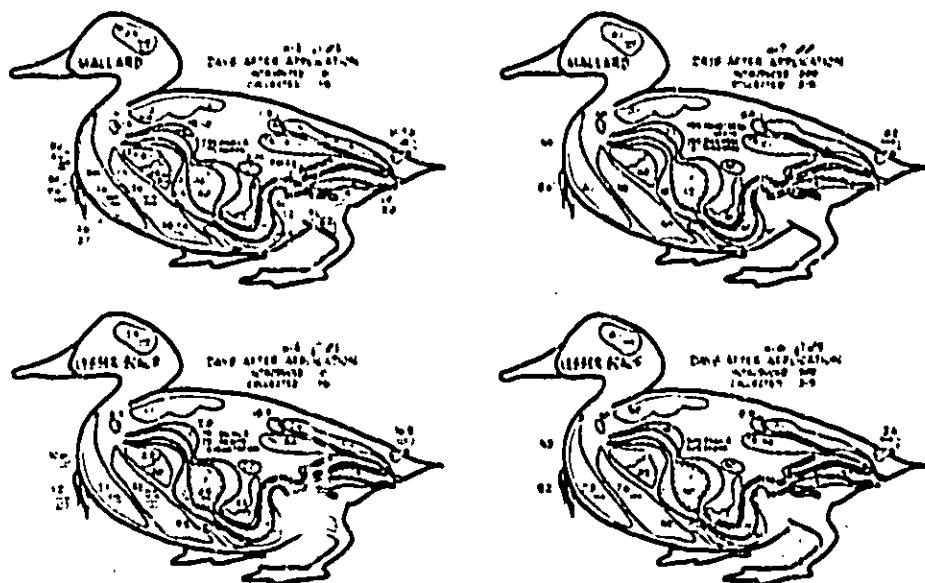


Fig. 1. Relative concentration of DDT residues (mean, ppm) in tissues^a of ducks exposed to a 4 acre/mash treated with 0.2 lb DDT per acre; species comparison.

^aTissue Legend

A adrenal glands	FH heart fat	H heart muscle	P pancreas
B brain	FL leg fat	K kidney	PR proventriculus
BF breast feathers	FM mesenteric fat	L lung	S spleen
BMA breast muscle	FP post ventral body fat	LC contents of large intestine	SC contents of small intestine
BS breast skin	G gizzard muscle	LI large intestine	T thyroid
D duodenum	GB gall bladder	LV liver	TE testes
FB breast fat	GC gizzard contents	OV ovary	U uropygial gland
		ND not detected	

transferring residues could account for several negative assays during the abundance of duckweed. The second possibility is that the DDT residues originated from the uropygial gland through preening. There is some contradictory evidence regarding the role of this gland in preening (Rawles 1960; Elder 1954). Maximum residue concentrations in feathers correspond to relatively high levels in uropygial glands. This could be a mode of excretion for fat-related pesticides. Perhaps residue concentrations of the gland could be dissipated by spreading the pesticide-lipid material over the feathers.

Breast Skin.—High residue levels in fat

storage tissues correspond to high concentrations in breast skin. Rawles (1960) describes the presence of fat accumulations and their associations with a subcutaneous network of accumulation in the dermis or corium of birds. Therefore, breast skin levels are high due to this microscopic accumulation of adipose cells in close association with the ducks' skin.

Uropygial Gland and Adipose Tissue.—In both scaup and mallards, the residue concentrations of lipids from uropygial glands are identical with residue levels of the post-ventral body fat. A sample collection method could be developed to obtain lipids from the uropygial glands of

living birds so that correlations may be made of post-ventral fat as well as other tissues. I was unsuccessful in my attempts to collect the lipid secretion with a capillary tube from living birds.

Immediately after exposure to DDT, the residues in different anatomical sites of fat deposition may vary by a factor of 30 (Table 11). This should cause some concern for those who indiscriminately select "fat" for pesticide analysis.

The highest concentration recovered from any tissue was 67.2 ppm in subcutaneous leg fat within the first 30-day exposure period. In addition, subcutaneous leg fat and neck fat in both species were two to four times greater than concentrations in the next highest organ, the uropygial gland. Other sites of fat deposition had residues either equal to or less than the uropygial gland levels. Fat samples taken after this period all contained concentrations lower than other tissues. Seasonal variation, food availability, and physiological activity (Rawles 1960) made fat samples not always accessible, and therefore, a total picture of the dynamics of DDT residues related to fat deposits can not be made.

A more complete sampling of lipid material was possible by using the uropygial gland, because fluctuations due to environment and physiology were not as apparent. With the exception of leg and neck fat, as seen above, residues of DDT in uropygial glands of both species were generally and consistently higher than all other tissues.

Residues in uropygial glands, fat depots, and adrenal glands follow similar dynamic trends related to exposure periods.

Brain.—Bernard (1963) suggested that as fat depots are utilized, DDT residues are recycled within the bird's system and accumulate in more sensitive areas, such as the brain. During November and December, 1961, when food was low and the win-

ter stress was high, a definite residue increase occurred in the brain. In May of the second year, food was very scarce, as indicated by weight loss of the duck. A definite residue increase occurred in liver, brain, and uropygial gland.

Adrenal Gland.—The adrenal glands exhibited some very high residue concentrations. Metabolites must have an affinity for adreno-cortical steroids of the interrenal (cortical) tissue. Bernard's (1963) suggestion of metabolite recycling and reaccumulation in sensitive areas may be illustrated in mallard adrenals. All of the highest residue concentrations were registered during the December, 1961, winter stress period.

Thyroid.—This gland never contained very low amounts of residue. The maximum levels occurred only during the initial 30-day exposure period of 1961. The mallard retained a maximum of 0.02 ppm in 1963, but the scamp had no detectable residues present. No definite lipid concentrations exist in this gland where residues could readily accumulate.

Testes.—No detectable DDT residue amounts were measured in the lesser scamp for the 2 years of the study (Table 10). Only one mallard testis was analyzed by ECCC. DDE (0.8 ppm) was the only metabolite found.

Ovary.—The maximum residue levels for mallards were almost identical to those concentrations in mallard testes (Table 10). Five samples of mallard ovary were analyzed by ECCC. The following metabolites were found: DDDMU, ND, DDE 0.7 ppm, DDD 0.1 ppm, *p,p'*-DDT 0.2 ppm.

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**IMAGE EVALUATION
TEST TARGET (MT-2)**

POINT
SIZE

4	5	6	7	8	9	10	11	12	13	14	15	16
150mm	150mm	150mm	150mm	150mm	150mm	150mm	150mm	150mm	150mm	150mm	150mm	150mm
Y0r8j A7o7q	Zl06x Ge92	7n34a K2b8t	D65mh 95c9d	2x5iy Gmn0c	Wcuzl L1cdg	6Y3si Okjdg	Futura	NEWS GOTHIc	K2bb8t Okjdg	4ef8k Gmn0c	7n34a K2b8t	Y0r8j A7o7q
9559u Licdg	339qn E119g	SuLli 339qn Oeh1 4e18s	Y555o El19g									9559u Licdg

150mm

6"

1.0	1.1	1.25	1.4	1.6	1.8	2.0	2.2	2.5
150mm								
125x 9559u Licdg								

4"

SERIF (BASKERVILLE) SIZE SANS SERIF (MICROFONT)

O27h9 B5c1P Wogbnk 81izx14C9E83 B18A J27QYI OKJDA

GjJliu Zm79a 3s13l 1skry 126FRKM EBTAU FUDWM BOONE

KP7x6 Rlml0t Umlk9t Eeard 10 VILNU MZHSF XMRBD HMEKH

Tlmh2 shjgjz hmlrt dmd3s 8 XHBD0 HMEKH XYHD SIDS

125x 9559u Licdg 125x 9559u Licdg 125x 9559u Licdg

5"



POINT
SIZE

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FILE SOME PAGES CONTAINING
MUTILATIONS AND OTHER DEFECTS
THESE UNAVOIDABLY CONSTITUTE
PART OF THE FILMED FILE.**

DUE TO POOR CONTRAST, SOME TEXT WRITTEN IN PENCIL MAY NOT BE LEGIBLE ON MICROFILM